Studies on the Renin-Angiotensin System in Hypertensive Patients

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IN THE following communication the results of two different studies on the renin-angiotensin system in patients with various types of hypertension will be presented.

PART I

The first study involved 101 arterial angiotensin determinations in 84 normal subjects and patients with various types of hypertension, using the method described by Boucher, Biron and Genest[1] in 1961. The results of these determinations are recorded in Table I.

Patients with hypertension associated with renal artery stenosis were divided into two sub-groups, depending on whether the blood was drawn from an artery or from the renal vein of the affected kidney. Twelve arterial determinations were performed on eight patients with renal artery stenosis; the average angiotensin concentration in this group was 51 ng.%. The difference between this mean value and that of the normal group is significant ($P < 0.01$). However, even in this group of patients, only 35% had arterial angiotensin levels above the upper limits of normal.

TABLE I.—ARTERIAL ANGIOTENSIN LEVELS* IN HUMAN HYPERTENSION

	No. of determinations	*Mean ± S.E.*	*"p"*	*No. of determinations above normal range (0-55 ng.%)*
Normal subjects (27)	28	7 ± 2		
Essential hypertension (25)	35	30 ± 8	0.05	7 (20%)
Malignant hypertension (8)	10	15 ± 7	Not significant	1
Hypertension associated with renal artery stenosis (8)	12	51 ± 21	<0.01	4 (35%)
Idem—renal venous blood (10)	10	62 ± 27	<0.01	3
Primary aldosteronism (6)	6	9	—	0

* Nanograms per 100 ml. blood.

Renal venous blood drawn from patients with hypertension associated with renal artery stenosis was taken at time of operation.

We wish to extend our thanks to Prof. Jérôme Conn and Dr. Maurice Verdy for their collaboration in providing us with blood from three of their patients with primary aldosteronism.

The mean level of 28 determinations of arterial angiotensin in 27 normal subjects was 7 nanograms per 100 ml. of blood (hereafter designated as ng.%). In 19 instances angiotensin was not detectable. One subject had an arterial angiotensin value of 55 ng.%.

Thirty-five determinations were carried out on 25 patients with essential hypertension; the mean arterial angiotensin level in this series was 30 ng.%. Although there was a significant ($p = 0.05$) increase in mean arterial angiotensin level in the group of patients with essential hypertension as compared to that of normal subjects, only 20% had levels above the normal range. The average arterial concentration in eight patients with malignant hypertension was 15 ng.%. Nine of the 10 determinations in this latter group were below 30 ng.%.

Ten patients with renal artery stenosis had angiotensin determinations on renal venous blood, taken at the time of surgery. The mean concentration in this group was 62 ng.%.

Of four patients with hypertension secondary to renal parenchymatous disease, only one patient with acute glomerulonephritis had a higher than normal arterial angiotensin level (100 ng.%). Four of six patients with proved primary aldosteronism had no detectable angiotensin in their arterial blood, and

TABLE II.—COMPARISON OF RESULTS OF ARTERIAL ANGIOTENSIN DETERMINATIONS OBTAINED BY DIFFERENT GROUPS OF WORKERS, IN PATIENTS WITH VARIOUS TYPES OF HYPERTENSION.

	Kahn *et al.*,[3] *1952*	Genest *et al.*,[1] *1961*	Morris *et al.*,[4] *1962*
Essential hypertension	2 patients (out of 18): above normal range $p = 0.03$	5 patients (out of 25): above normal range $p = 0.05$	0 (20 patients)
Malignant hypertension	Average: 20 times the mean normal value (10 patients)*	0 (8 patients)	0 (5 patients)
Renovascular hypertension		35% above normal range (10 patients) $p < 0.01$	↑ ↑ ** (13 patients)

* "Many of them had some degree of congestive heart failure" (*J. Exp. Med.*, 95: 527, 1952).
** Similar findings obtained by Langford (1961).

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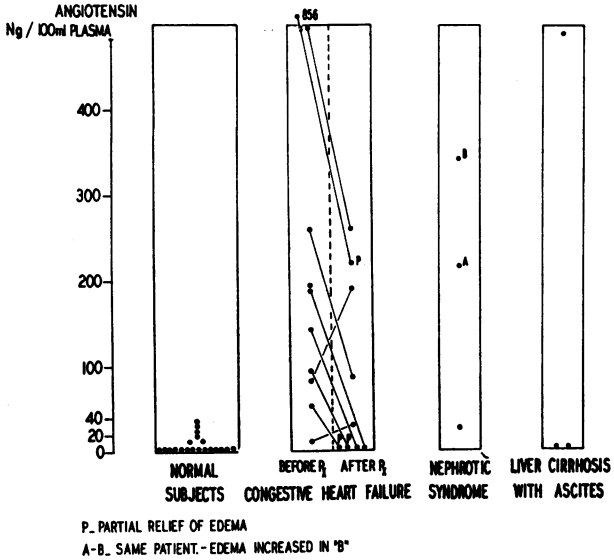

Fig. 1.—Arterial angiotensin levels in normal subjects and edematous patients. Two patients in the congestive heart failure group who showed an increase in angiotensin levels following total relief of edema were also hypertensive. All other patients in this group were normotensive and their heart failure was secondary to valvular or arteriosclerotic heart disease.

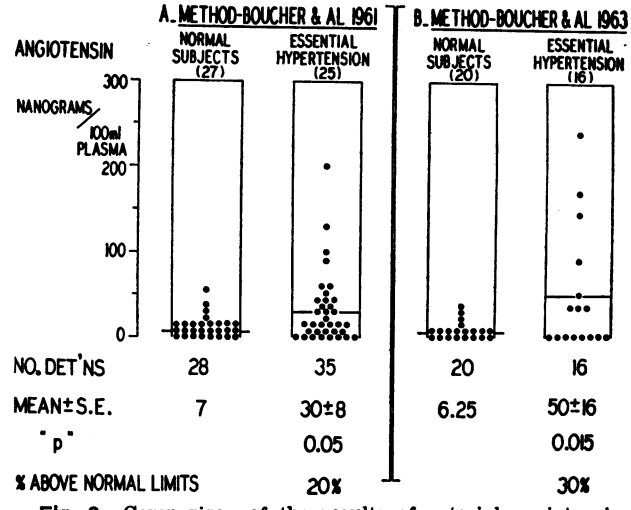

Fig. 2.—Comparison of the results of arterial angiotensin levels in patients with essential hypertension, obtained (a) by means of the method described by Boucher *et al.* in 1961 and (b) by the new procedure described in this Symposium. It is obvious that only a minority of patients with essential hypertension have excessive angiotensin levels, despite the significance of the mean difference.

two others had levels of 20 and 30 ng.%.

These results strongly suggest that increased circulating arterial angiotensin levels are not the cause of the maintenance of increased diastolic pressure in the majority of patients studied. These observations are in agreement with the findings obtained in dogs with experimental hypertension by Scornik and Paladini.[2]

As illustrated in Table II, our findings in patients with essential hypertension are in fairly close agreement with those obtained by Kahn *et al.*,[3] whereas Morris, Ransom and Howard[4] did not detect any angiotensin in arterial blood from similar patients. Our findings in patients with renovascular hypertension did not show the same frequency of high angiotensin levels as those reported by Morris, Ransom and Howard[4] and by Langford and Day[5] in similar patients. Our results in patients with malignant hypertension are in agreement with those described by Morris, Ransom and Howard[4] but differ from those reported by Kahn *et al.*[3] in 1952. The latter authors stated that a number of their 10 patients with malignant hypertension had "some degree of congestive heart failure".[3] The presence of this complication in most of the patients studied by Kahn *et al.* invalidates their interpretation of such results, as far as their relation to malignant hypertension is concerned. These are more in agreement with those which we have obtained in normotensive patients with congestive heart failure and edema.[6]

Our findings in a study of arterial angiotensin levels in 15 patients with generalized edema are illustrated in Fig. 1. In 10 instances, the edema was due to congestive heart failure secondary to valvular or arteriosclerotic heart disease in eight patients and to hypertensive cardiovascular disease in two. Nine of these patients were studied both

TABLE III.—ESSENTIAL HYPERTENSION

*Patients**	*Age*	*Sex*	*B.P. Admission*	*B.P. Time of sampling*	*Angiotensin** (nanograms %)*
P.R.	42	F	220/150	220/150	90
I.V.	35	M	150/90		0
D.R.	37	F	162/110	196/110	0
G.D.	47	M	210/120	190/120	0
M.N.	45	M	220/130	160/110	145
G.C.	57	M	180/120	150/96	50
M.V.	20	F	172/110	170/102	35
R.G.	24	F	175/115	160/105	0
R.G.	57	M	170/110	170/90	170
R.D.	36	M	160/100		35
A.P.	65	F	210/115	185/90	35
E.L.	44	M	250/130	180/130	0
M.P.	24	F	200/160	200/130	240
R.G.	28	M	196/130	280/145	0
R.M.	20	M	200/150	180/110	0
H.L.	40	M	178/130	150/112	0
					Mean: 50
					p: 0.015 (as compared to group of normal subjects)

* Unrestricted diets.
** New procedure of Boucher, Biron and Genest.[1]

TABLE IV.—HYPERTENSION ASSOCIATED WITH RENAL ARTERY STENOSIS

Group A—No gradient of pressure beyond stenosis

Patients	*Age*	*Sex*		*B.P. Admission*	*B.P. Time of sampling*	*Angiotensin**	*Renin activity***
K.B.	48	F	Bilateral fibromuscular hyperplasia	210/130	150/90	Art. 0 Rt. R.V. 0 Lf. R.V. 0	
W.M.	36	M	Bilateral R.A.S.	210/130	210/140	Art. 0	220 255
A.D.	38	F	Bilateral R.A.S. Malignant hypertension	244/150	210/118	Art. 0 Rt. R.V. 0 Lf. R.V. 0	
G.D.	59	F	Left R.A.S. Marked hyperaldosteronism Malignant hypertension	240/130	230/120	145	10,000

* Nanograms per 100 ml. of plasma.
** Nanograms of angiotensin—3 hours' incubation.

before and after total or partial relief of edema following administration of natriuretic agents. During the period of congestive heart failure and edema, nine of these patients had greatly increased arterial angiotensin levels, up to 856 ng.%. After total or partial relief of edema, angiotensin completely disappeared from the arterial blood of four of these, and in three others there was a marked decrease. In one patient there was no significant change, and in another a marked increase was observed. It is of interest to note that in these two last patients the congestive heart failure was secondary to hypertensive cardiovascular disease.

The apparent discrepancy between the normal or undetectable levels of arterial angiotensin in patients with malignant hypertension and the high renin activity first reported in such patients by Judson and Helmer[7] and Helmer[8, 9] may be explained by studies described in the second part of this communication.

PART II

In the second part of this study arterial angiotensin and renin activity levels were determined by the new procedures described earlier in this Symposium by Boucher, Biron and Genest.[1] These techniques were employed: (1) in a second study of arterial angiotensin levels in a group of 16 patients with essential hypertension, and (2) in a research project concerned with the mechanism of hypertension in patients with renovascular hypertension.

TABLE V.—HYPERTENSION ASSOCIATED WITH RENAL ARTERY STENOSIS

Group B-I—"Significant" pressure gradient

Patients	*Age*	*Sex*		*B.P. Admission*	*B.P. Time of sampling*	*Gradient (systolic)*	*Angiotensin**	*Renin activity***
M.F.	49	M	Bilateral R.A.S.	220/140	140/80	70	Art. 0 Lf. R.V. 0	
J-P.R.	45	M	Bilateral R.A.S.	220/140	192/110	34	Art. 0 Lf. R.V. 0	375
P-E.R.	41	F	Bilateral R.A.S.	240/120	180/100	L 106 R 86	0	125
H.P.	49	M	Left R.A.S.	240/150	230/130		0	750
				At operation		70	0	835

* Nanograms per 100 ml. of plasma.
** Nanograms of angiotensin—3 hours' incubation.

TABLE VI.—HYPERTENSION ASSOCIATED WITH RENAL ARTERY STENOSIS

Group B-II—"Significant" pressure gradient

Patients	*Age*	*Sex*		*B.P. Admission*	*Time of sampling*	*Gradient (systolic)*	*Angiotensin**	*Renin activity***	*JGC counts and % cells types 2 & 3*
A.deP.	48	F	Right fibromuscular hyperplasia	185/115	170/120	80	Art. 110 Rt. R.V. 40		
A.R.	40	M	Left R.A.S.	180/115	140/100	100	Art. 35 Lf. R.V. 80	600 3700	361 (6%)
R.P.	37	M	Left R.A.S.	242/150	240/160	100	Art. 30 Lf. R.V. 135	280 20,000	440 (14%)
A.R.	51	F	Left R.A.S.	180/110	120/80	60	80	375	
J.M.	13	F	Right R.A.S.	170/120		total		550	427 (17%)

* Nanograms per 100 ml. of plasma.
** Nanograms of angiotensin—3 hours' incubation.

TABLE VII.—HYPERTENSION ASSOCIATED WITH RENAL ARTERY STENOSIS

Group C—Pressure gradient unknown

Patients	*Age*	*Sex*		*B.P. Admission*	*B.P. Time of sampling*	*Angiotensin**	*Renin activity***
D.H.....	50	M	Right R.A.S.	260/170	250/160	45	
R.G.	40	M	Left R.A.S.	170/100	180/125	550	1380
P.B.	35	F	Bilateral fibromuscular hyperplasia	180/140	170/110		250
E.V.	58	M	Right R.A.S. Left R.A. thrombosis	180/105	160/112	40	3025
M.C.	35	M	Renal hypoplasia with pyelonephritis	190/140	190/100	170	
M.D'H.	59	M	Bilateral R.A.S.	168/110	175/110	0	

* Nanograms per 100 ml. of plasma.
** Nanograms of angiotensin—3 hours' incubation.

The results of this second study of arterial angiotensin levels (Table III) in 16 patients with essential hypertension essentially confirm those described in Part I of this report. The results obtained by the two procedures, both in normal subjects and in patients with essential hypertension, are compared in Fig. 2. It is of interest to note that exactly the same proportion (one-half) of patients with essential hypertension in both series had no detectable levels of angiotensin in their arterial blood.

The study of the mechanism of hypertension in patients with renal artery stenosis (hereafter designated as R.A.S.) was started six months before the preparation of this report and involved the measurement of the following parameters: (1) sodium metabolic balance under conditions of fixed intake of sodium and potassium, (2) repeated determinations of aldosterone excretion and/or secretion rate, (3) estimations of arterial and renal venous angiotensin and renin activity levels, (4) measurement of pressure gradients, either by catheter guided beyond the renal stenosis at the time of renal angiography, or taken at time of surgery, (5) serial determinations of serum electrolytes, intravenous pyelography and renal angiography, (6) measurements of blood and plasma volumes and (7) juxtaglomerular cell counts and calculation of the percentage of cells of types 2 and 3 (granular cells).

Nineteen patients with hypertension associated with R.A.S. have been studied more or less completely by the above-noted procedures. These patients have been divided into four sub-groups: (a) four patients showing no pressure gradient beyond the stenosis (Group A, Table IV), (b) four patients with a significant pressure gradient but without any detectable arterial angiotensin (Group B-I, Table V), (c) five patients with a significant pressure gradient and with increased arterial angiotensin levels (Group B-II, Table VI), and (d) six patients whose pressure gradients are not known (Group C, Table VII).

In the first group (Group A, Table IV), the findings indicate that in three patients without any pressure gradient beyond the stenosis there was no detectable arterial or renal venous angiotensin level, and that there was a normal level of arterial renin activity, as measured twice in patient W.M. Only patient G.D. presented very high arterial angiotensin and renin activity levels. This patient had a slight left renal artery stenosis as shown by angiography, but showed no pressure gradient. She presented a syndrome typical of primary hyperaldosteronism and severe malignant hypertension accompanied by necrotizing arteriolitis and diffuse ischemic tubular atrophy of both kidneys (as revealed later by autopsy). It is of interest to compare the findings in the arterial, and left and right renal venous blood obtained at the time of surgery (Fig. 3). As illustrated in this figure, the renin activity levels were very high, especially in the right renal venous blood, whereas the angiotensin levels were undetectable in the left renal venous blood, normal in the arterial blood and only slightly increased in the right renal venous blood. The

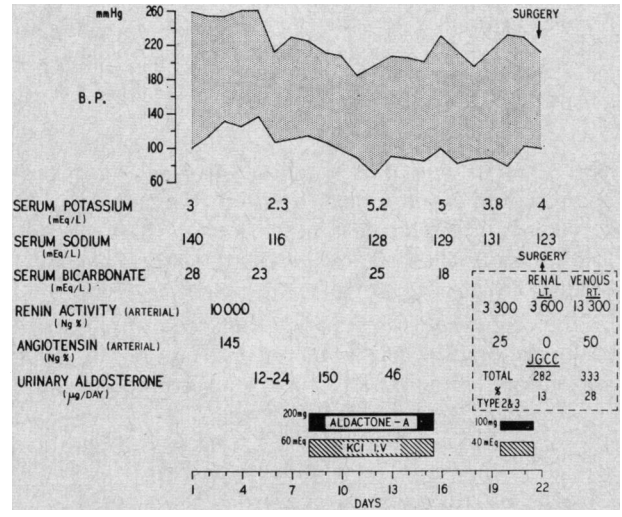

Fig. 3.—G.B., a 50-year-old patient, was referred with a syndrome of primary hyperaldosteronism (serum potassium levels below 3 mEq./day, marked muscular weakness, paresthesias, nocturia), malignant hypertension and moderately advanced renal failure. The blood pressure readings recorded for each day are the average of 14 hourly readings. (Same in following figures.) Proteinuria varied from levels of 100 mg. to 2.6 g./day. There was an abdominal Grade II/IV systolic murmur best heard at the left of the umbilicus. The urinary aldosterone levels of 12 and 24 µg./day (measured by the procedure of Nowaczynski, Koiw and Genest[10]) are very slightly elevated in contrast to the high circulating angiotensin level of 145 ng. %. The high renin activity in arterial and in renal venous blood obtained from both kidneys at the time of surgery is well correlated with the juxtaglomerular cell counts of 282 and 332 and the high percentage of cells of types 2 and 3. The identification of the factors which prevent the liberation of angiotensin in the presence of such high levels of renin activity would be of great importance.

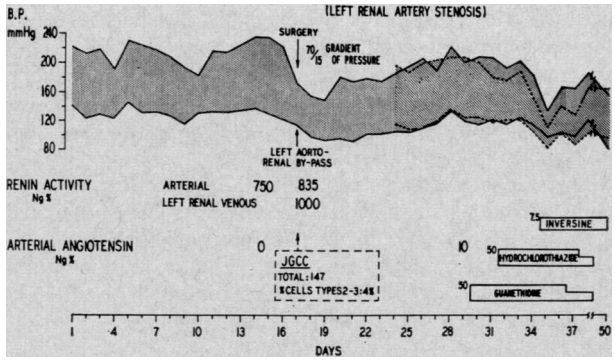

Fig. 4.—Despite the significant pressure gradient, this patient, H.P., had no detectable angiotensin in arterial blood, although the arterial activity and left renal venous activity were increased two- to three-fold. The lack of success of the bypass procedure by a venous graft (which was patent, as confirmed by postoperative renal angiogram) is in accord with the absence of circulating angiotensin, the low juxtaglomerular cell count and the normal proportion of cells of types 2 and 3. This patient was hypertensive for the last five or six years and had no family history of hypertension. His serum potassium was 4.4 mEq./l. and his serum bicarbonate was 24 mEq./l.

high renin activity coincided with the increased juxtaglomerular cell count and the high percentage of cells of types 2 and 3. These findings, and others described below, reconcile our observations on angiotensin with those of Judson and Helmer[7-9] and of Fitz and Armstrong[11] on renin activity and illustrate the complexity of the renin-substrate reaction.

The mechanism of hypertension is difficult to understand in the second group of patients with significant pressure gradients but without any increase in arterial or renal venous angiotensin levels (Group B-I, Table V). It is of interest to note in patient H.P. the two- to three-fold increase in arterial renin activity and the absence of any detectable angiotensin in arterial blood. The findings in this group (Group B-I, Table V) suggest that the renal artery stenoses noticed on the angiograms and confirmed at surgery were not the cause of the hypertensive process but the consequences of the accelerated atherosclerotic process. Such a possibility is illustrated by Fig. 4, which depicts our findings in the case of patient H.P., in whom successful bypass of the stenotic area in the renal artery by a venous graft (confirmed by a postoperative renal angiogram) was not accompanied by any significant improvement in the hypertensive condition (at least up to three months after operation). The presence of a low juxtaglomerular cell count with a normal ratio of cells of types 2 and 3, the absence of arterial angiotensin and the lack of benefit following surgery confirm previous observations made by Turgeon and Sommers[12] and by Crocker *et al.*,[13] Itskovitz *et al.*[14] and Goldberg and McCurdy[15] in similar patients.

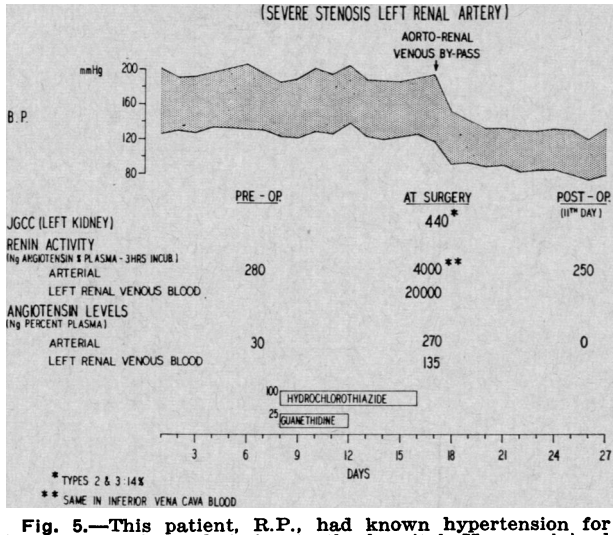

Fig. 5.—This patient, R.P., had known hypertension for 10 months before admission to the hospital. He complained of weakness and paresthesias. A Grade II/IV abdominal murmur was heard over the left periumbilical area. Serum potassium was 4.6 mEq./l. and serum bicarbonate 27.5 mEq./l. It is of interest to note the normal arterial angiotensin and renin-activity levels in the preoperative period, despite a high juxtaglomerular cell count and a markedly increased proportion of cells of types 2 and 3. At the time of operation these two parameters were greatly increased in both arterial and left renal venous blood. This may be due, at least in part, to the previous administration of hydrochlorothiazide, as is demonstrated in the presentation by Veyrat *et al.* elsewhere in this Symposium.

In the third group of patients with significant pressure gradients and a high arterial angiotensin level (Group B-II, Table VI), there was a good correlation between the juxtaglomerular cell counts and the proportions of cells of types 2 and 3, on the one hand, and the high renin activity and angiotensin levels on the other hand, although the correlation of these parameters showed considerable variations. For example, in patient R.P. (Fig. 5) the arterial angiotensin and renin activity levels in the preoperative period were within normal limits, with a high juxtaglomerular cell count and a 14% proportion of cells of types 2 and 3. The very high renin activity and angiotensin levels found at the time of surgery in arterial and left renal venous blood may be due, at least in part, to the previous administration of hydrochlorothiazide, given to reduce the high blood pressure levels before operation.

The findings recorded in Table VII (Group C) concerning the last group of patients with unknown pressure gradients show wide variations in the relative proportions of angiotensin to renin activity between the various patients. Patient R.G. had a plasma arterial angiotensin of 550 ng. % whereas his renin activity was only 1380 ng. % of angiotensin per three hours of incubation. This is in contrast to the findings in patient E.V., who had an arterial angiotensin of 40 ng.% with a renin activity of 3025 ng.% of angiotensin released during three hours' incubation.

These observations appear to indicate that the *in vivo* liberation of angiotensin varies greatly from patient to patient, and in the same patient depending on the state of his illness. They also emphasize our ignorance concerning the *in vivo* kinetics of the enzyme-forming and enzyme-destroying angiotensin systems and of the factors controlling the levels of circulating angiotensin (Fig. 6). There is a great need for a more fundamental understanding of the kinetics of the various

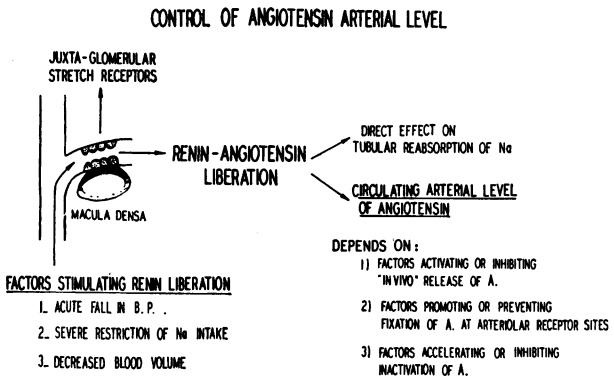

Fig. 6.—Control of angiotensin arterial level. Illustrating one of the factors stimulating renin liberation by the juxtaglomerular cells, and regulating the circulating arterial angiotensin levels. Other possible factors, such as those involved in the inactivation of renin and in the control of the converting enzymes, must also be studied.

systems and the factors involved in angiotensin formation, binding by receptors, and destruction.

In serial studies of urinary aldosterone and arterial angiotensin levels in patients with various types of hypertension, we have not detected any correlation between these two parameters.[16] Such findings underline again the fact that, under usual conditions, aldosterone secretion is not unidependent on angiotensin, but is the end result of many other influences which may limit the action of the liberated angiotensin. It should also be remembered that renin itself has no direct action on aldosterone secretion when infused directly into the adrenal artery.[17]

SUMMARY

Results of determinations of arterial angiotensin and renin activity levels obtained in our laboratory on patients with various types of hypertension have been presented. In two different studies using the two procedures described by Boucher, Biron and Genest[1] for measurement of blood angiotensin, we have demonstrated that the maintenance of increased diastolic pressure in the majority of hypertensive patients studied, whether the hypertension was of the essential, malignant or renovascular variety, is not caused by high levels of circulating arterial angiotensin. Significant variations in the relative amounts of angiotensin and the activity of renin in different patients have been observed and suggest the need of more fundamental studies to provide a better understanding of the factors regulating the circulating angiotensin levels.

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